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Mutagenicity of 2,4,6-Trinitrotoluene-Surfactant Complexes

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Environmental hazards result from 2,4,6-trinitrotoluene (TNT) contamination of waters, soils and sediments by wastes from munitions manufacturing, loading, assembling and packing facilities. TNT has been identified in soils (KLAUSMEIER et al. 1973) and in the groundwater after leaching from disposal sites (PEREIRA et al. 1979).

TNT has been shown to cause liver damage and anemia in humans (SAX 1963). It is toxic to rats, mice (LEE et al. 1975), fish (OSMON and KLAUSMEIER 1972; NAY et al. 1974), unicellular green algae, copepods and oyster larvae (WON et al. 1971). TNT also inhibits the growth of many fungi, yeasts, actinomycetes and Gram-positive bacteria (KLAUSMEIER et al. 1973). It is mutagenic in the Ames test (DILLEY et al. 1979).

The problems associated with the discharge of TNT into the environment have prompted investigations into a number of treatment alternatives to alleviate this hazard, including carbon adsorption, lagoon storage, photolysis and chemical treatments. Recent findings have indicated that certain amino surfactants under alkaline conditions rapidly complex TNT to form a water insoluble, nonexplosive precipitate which can be filtered out of solution and incinerated or land-filled. (OKAMOTO and WANG 1977; CROCE and OKAMOTO 1978; OKAMOTO et al. 1978).

This reaction appeared feasible for an on-line process for the purification of wastewater contaminated with TNT, since only traces of TNT were found after treatment and filtering. The process has also been proposed for the *in situ* treatment of contaminated lagoons and soils. The same surfactant treatment is also effective with other munition wastes, hydrolyzing hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX), octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX) and nitroglycerine (CROCE and OKAMOTO 1978). An insoluble complex is formed with 2,4-dinitrotoluene.

It is the purpose of this report to assess the mutagenicity of these TNT-surfactant complexes and the filtrate which results after the complex has been removed from the waste stream.

METHODS

Commercially available TNT (Eastman Kodak, Rochester, NY) was recrystallized. The surfactants Duoquad T-50 (N-tallow-N'N'N'-trimethyl-N,N-dimethyl-1,3-diamino-propane, 50% active solution), Duomeen T (N-tallow-1.3-diamino-propane, white waxy solid) and

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Arquad T-50 (N-tallow-trimethylammonium surfactant, 50% active solution) and the corresponding TNT-surfactant complexes were supplied by Dr. Y. Okamoto, Polytechnic Institute of NY. The surfactants are commercially available from Armak Corp. McCook, IL.

A sample of filtrate from a Composition B (40% TNT, 60% RDX) waste stream which had been treated with Duoquad T-50 at pH 11.0 and filtered in a pilot scale facility was used to test the water quality of the final filtrate from this process. The pH of the filtrate was adjusted to pH 7.0 prior to testing.

The Ames screening test for mutagenicity was performed according to standard procedures (AMES et al., 1975). The surfactants (Duoquad T-50, Arquad T-50 and Duomeen T), TNT and the three TNT-surfactant complexes were tested. Five strains of *Salmonella typhimurium* (TA98, TA100, TA1535, TA1537, TA1538), were used to test these compounds over a range of concentrations with and without metabolic activation (S9). The tests were run in triplicate and a three-fold increase in back mutations was considered as the criterion for a positive test for mutagenicity. The Composition B filtrate sample was diluted and concentrated to evaluate the response over a range of concentrations. Concentration was accomplished by rotary evaporation at 45°C. The solution changed from yellow to orange when concentrated.

Methylene chloride was selected as solvent for the Duomeen T-50-TNT complex in the Ames test, since the complex is not soluble in DMSO. Methylene chloride is mutagenic to TA98, but does not affect the other strains. Formamide, dimethylformamide, 1-methyl-2-pyrrolidinone, tetrahydrofurfuryl alcohol, tetrahydrofuran and acetonitrile were also examined as possible solvents, but were rejected because of complex insolubility or mutagenic/toxic activity to the test strains.

RESULTS

All three complexes were toxic to the test organisms at a concentration of 5000 µg per plate. The dose response curves for the complexes are presented in Figs. 1,2 and 3. On all figures the horizontal line represents the line of significance for mutagenic activity. The three compounds produced higher mutation rates than TNT at equivalent concentrations. TNT was mutagenic to TA1538, TA98 and TA100, while the Duoquad-TNT complex was mutagenic to all five strains and at lower concentrations than TNT; 0.5 µg/plate with TA1538 and 5 µg/plate with TA98 without S9, compared to 50 and 500 µg/plate for TNT. The Arquad-TNT complex was mutagenic to TA1537, TA1538 and TA98 at concentrations as low as 5 µg/plate with TA1538 with and without S9, and TA98 without S9. The Duomeen-TNT complex was also mutagenic to TA1537 and TA1538; at 5 µg/plate with TA1538 and S9. For the Duomeen-TNT complex, the solvent methylene chloride was mutagenic to TA98, while TNT tested in this solvent was mutagenic to TA1537 and TA1538. The surfactants, Duomeen T and Duoquad T-50 were not mutagenic, while Arquad T-50 tested positive at 50 µg/plate with TA1538 with and without S9. The surfactants were toxic at concentrations of 50 µg/plate with Duomeen T, and 500 µg/plate with Arquad T-50 and Duoquad T-50.

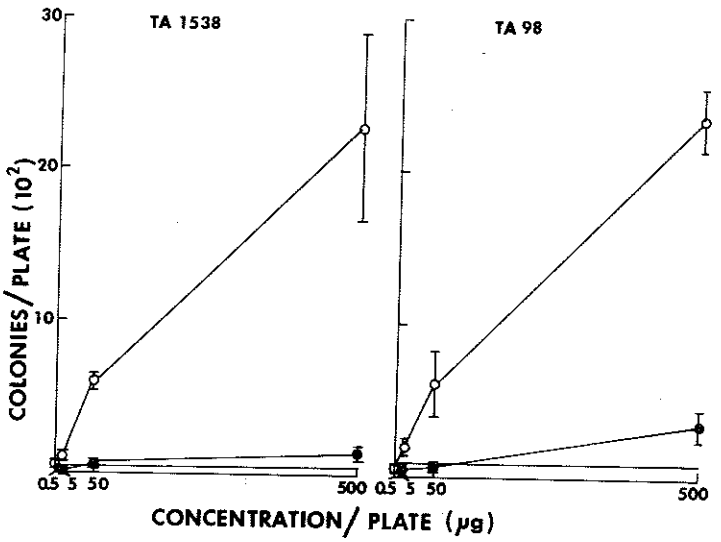

Figure 1. Dose response of TA1538 and TA98 to TNT (●) and Duoquad T-50-TNT complex (O) without S9. Levels of significance are above 33 and 75 colonies/plate for TA1538 and TA98, respectively.

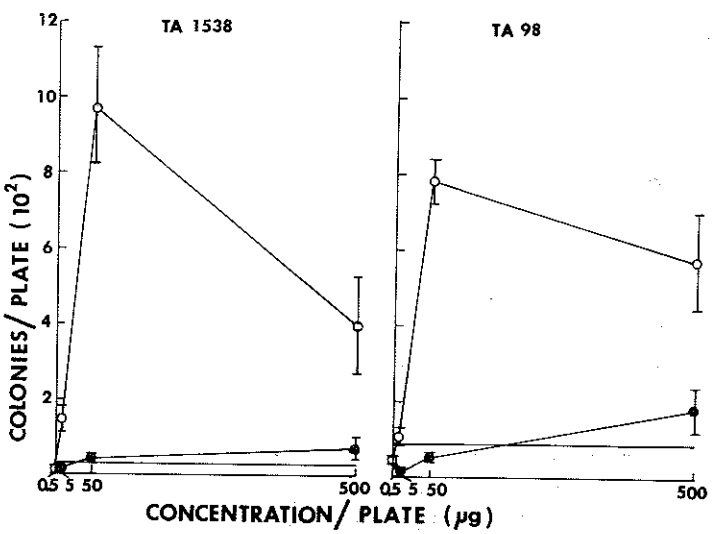

Figure 2. Dose response of TA1538 and TA98 to TNT (●) and Arquad T-50-TNT complex (O) without S9. Levels of significance are above 33 and 87 colonies/plate for TA1538 and TA98, respectively.

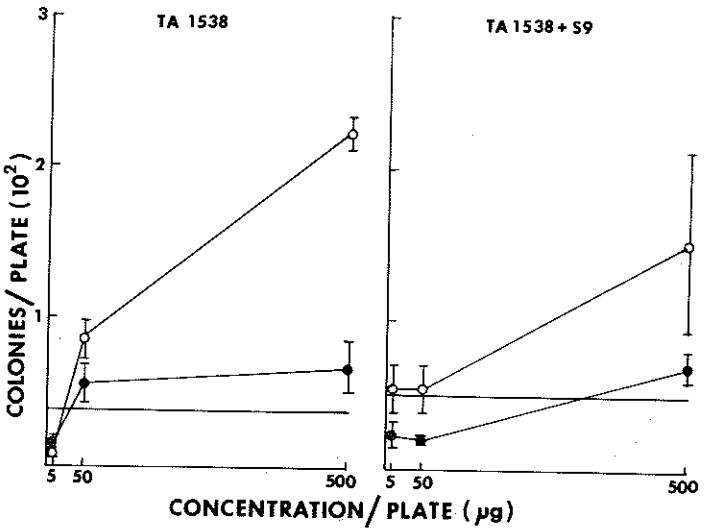

Figure 3. Dose response of TA1538 with and without S9 to TNT (●) and Duomeen T-TNT complex (O). Levels of significance are above 39 and 36 colonies/plate for TA1538 with and without S9, respectively.

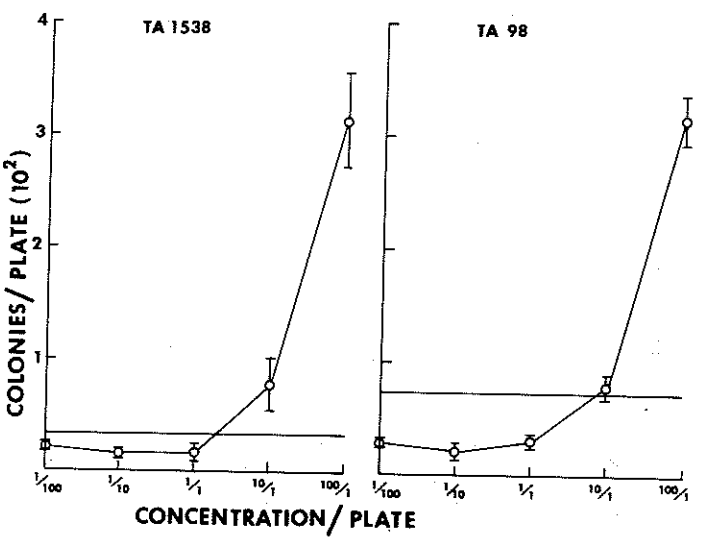

Figure 4. Dose response of TA1538 and TA98 to different concentrations of Composition B filtrate. Levels of significance are above 33 and 75 colonies/plate for TA1538 and TA98, respectively.

Figure 4 illustrates the responses of TA1538 and TA98 to the dilution series of the Composition B filtrate. At or below process concentration no mutagenic activity was found, but upon concentration, positive results were found with TA98, TA1535, TA1537 and TA1538.

DISCUSSION

There was an increase in mutation rate caused by the complexes, when compared with the rate resulting from exposure to TNT or the surfactants alone under the same experimental conditions. This synergistic effect occurs with all three complexes (Duoquad T-50-, Duomeen T- and Arquad T-50-TNT). The concentrations at which positive findings were detected were below the levels for TNT alone. The surfactants, Duoquad T-50 and Duomeen T, tested negative, but Arquad T-50 gave a positive result with TA1538 at 50 µg/plate. This may be due to the surfactant itself or impurities arising during its manufacture.

The toxicity of these surfactants reflects the biocidal nature of these compounds (HUECK et al. 1966), which limits the ability to test for mutagenicity at higher concentrations.

A Composition B waste stream which originally contained TNT, RDX and other residues from explosives manufacture, was treated with Duoquad T-50 at pH 11 and filtered. After concentration, the filtrate was significantly mutagenic to the test organisms. This illustrates the fact that the surfactant treatment did not entirely eliminate mutagens in this complex waste stream, even though it is known that the principal contaminants do undergo complexation or hydrolysis. The mutagenic agents in this case could be residual TNT or TNT-surfactant complex, hydrolysis products from RDX or other compounds formed during the treatment process. Further investigation is required to determine whether a potential hazard may develop as a result of alteration of process variables or concentration of the mutagenic contaminants by physical or biological processes in the biosphere.

The fact that the TNT-surfactant complexes present more of a hazard than TNT itself raises a concern whether soil burial is a suitable method of disposal of these complexes. Alternative methods should be investigated, including incineration.

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